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Microstimulation of the Lumbosacral Spinal Cord: Mapping

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INTRODUCTION

Electrical stimulation of the nervous system is a means to restore function to individuals with neurological disorders. The objective of this project is to investigate the feasibility of neural prosthetics based on microstimulation of the spinal cord with penetrating electrodes. The objectives of this project are to define the anatomical locations of spinal neuronal populations involved in control of genitourinary and motor functions, and to map the physiological responses evoked in the genitourinary and hindlimb motor systems by microstimulation in the lumbosacral spinal cord.

Specifically, we used chemical and viral retrograde tracers and immediate early gene expression to determine the locations in the spinal cord of the neuronal populations that control genitourinary and motor functions in the male cat. We use intraspinal microstimulation with penetrating activated iridium microelectrodes to determine the physiological effects of stimulation of different neural populations. The results of this project answer fundamental questions about microstimulation of the spinal cord, and may lead to development of a new generation of neural prosthetics for individuals with neurological impairments.

SUMMARY OF PROGRESS

Mapping the neural network innervating the bladder and urethra using pseudorabies virus

The CNS neurons that are involved in control the urinary bladder and proximal urethra were identified by retrograde transport of the transganglionic tracer cholera toxin β-subunit (CTb) or the viral transneural tracer pseudorabies virus (PRV, Bartha strain). CTb or PRV was injected into the wall of either the bladder or urethra of male Sprague-Dawley adult rats. After 7-10 days survival for CTb-injected animals or 3-7 days survival for PRV-injected animals, the spinal cords and brains were processed for immunocytochemical detection of CTb or PRV. CTb labeled cells were found in the intermediolateral region (IML) of L6-S1 and in the IML of L1-L2, corresponding respectively to the parasympathetic and sympathetic innervation of the bladder and urethra. At 72 h following injection of PRV into the wall of the urethra or the urinary bladder, PRV-infected (PRVi) neurons were observed in L6-S1 within the IML, dorsal commissure, around the central canal, and within the superficial layers of the dorsal horn. At 4 and 5 days post injection, dense PRVi neurons were found in the dorsal aspect of the pons in Barrington's nucleus, and fewer labeled neurons were observed in locus ceruleus, subceruleus nuclei, and the Kölliker-Fuse nucleus. In the ventral aspect of the pons, PRVi neurons were found within the nucleus raphe magnus and in the ventrolateral pontine norepinephrine containing neurons (A5). In the mesencephalon, PRVi neurons were found in the ventrolateral periaqueductal gray and in the gigantocellular division of the red nucleus. At post-injection days 5, 6, and 7, PRVi cells were observed in the lateral hypothalamus and paraventricular nucleus, and at days 6 and 7 in the retrochiasmic region and within the suprachiasmatic nucleus. In addition, at days 6 and 7 labeling was observed in the amygdaloid nuclei, lateral septal nucleus, hippocampus, frontal motor cortex, and piriform cortex. These results for the first time identify the higher supraspinal neural network that is involved in control of the lower urinary tract, and provide an anatomical framework for physiological studies.

Following our succesful studies in male rats, we conducted parallel studies in adult male cats. Fluorogold, cholera toxon b-subunit, and PRV were all used as retrograde tracer to map the spinal innervation of the preprostatic urethra in adult male cats. The Bartha strain of PRV was injected using aseptic technique into the preprostatic urethra of three adult immunologically naïve male cats that were raised from birth in isolation. After an 11 day post-injection survival period, the animals were perfused and the spinal cord was processed for immunocytochemical detection of PRV. No satifactory labeling was obtained using the Bartha strain of PRV in cats, although the same viron and processign produced consistent infection in rats. Therefore, we conclude that the Bartha strain of PRV is not an effective retrograde tracer in adult cats. Similarly, cholera toxin B-subunit and Fluorogold were injected into the preprostatic urethra of three male adult cats.

Following an 11-12 day post-injection survival period, the animals were perfused and the spinal cords processed for immunocytochemical detection of CTb. Neurons labeled following CTb injection into the pre-prostatic urethra were observed in the lateral aspect of the intermediolateral nucleus of the sacral spinal cord. The greatest density of neurons was found in S1 with significant numbers in the S2 segment, and small numbers of labeled neurons in caudal L7. The neurons were comparable to those observed following retrograde tracing of the innervation of the bladder.

Identification of spinal neurons active during reflex micturition

In these studies we used expression of Fos protein to identify cells within the spinal cord that regulate micturition in male cats. The immediate early gene c-fos that encodes the Fos protein can be induced rapidly and transiently in neurons by increased electrical activity. Animals were anesthetized with alpha-chloralose and received 1 of 4 stimulus protocols: electrical stimulation of the pelvic nerve, electrical stimulation of the pudendal nerve, a period of isometric micturition induced by ligating the proximal urethra and infusing saline into the bladder, or electrical stimulation of Barrington's nucleus. After the period of stimulation, the animals were perfused and neurons expressing Fos-like immunoreactivity (FLI) were visualized with immunocytochemical methods. Stimulation with each protocol resulted in a substantially larger number of neurons expressing FLI than in operated but unstimulated controls, which exhibited few Fos-positive neurons localized to the superficial dorsal horn. In animals undergoing isometric micturition or stimulation of Barrington's nucleus, neurons exhibiting FLI were found bilaterally in the sacral (S1-S3) spinal cord and were localized to the lateral portion of the superficial dorsal horn (laminae I and II), in the intermediolateral region (lateral laminae V-VII), and around the central canal (lamina X and medial laminae V-VII). The intermediolateral region appeared to contain two populations of cells exhibiting FLI: a group of large multipolar cells and a group of small round cells. Many fewer Fos immunoreactive nuclei were observed in the medial portion of the superficial dorsal horn and FLI was not observed in ventral horn neurons. Electrical stimulation of the pudendal or pelvic nerves resulted in fewer numbers of cells exhibiting FLI, with a less widespread spatial distribution. These results identify spinal neurons that are active during the micturition cycle, and demonstrate that a behaviorally relevant stimulus (isometric micturition) generated more widespread and greater intensity Fos expression than repetitive electrical stimulation of the component peripheral nerves.

Colocalization of parvalbumin and c-Fos in sacral spinal neurons involved in regulation of micturition

The purpose of this project was to determine the location of inhibitory spinal interneurons involved in regulation of the genitourinary system. We used expression of the *c-fos* gene encoded protein c-Fos to identify sacral spinal neurons that were active during reflex micturition, and co-localization with parvalbumin (PV), a calcium binding protein present in GABAergic neurons, to identify putative inhibitory neurons. Adult male cats were anesthetized with alpha-chloralose and underwent a 1-2 h period of isometric micturition induced by ligating the proximal urethra and infusing saline into the bladder (1 ml/min) until spontaneous periodic bladder contractions occurred. Animals were perfused 1-2 h after the cessation of the stimulus, and double labeling was used to define co-expression of c-Fos with PV or GABA. Operated unstimulated controls exhibited few neurons expressing c-Fos which were localized to the superficial dorsal horn (laminae I and II). In stimulated animals, neurons expressing c-Fos were found bilaterally in S1-S3 and were localized to the lateral portion of the superficial dorsal horn (laminae I and II), the intermediolateral region (lateral laminae V-VII), and around the central canal (lamina X and medial laminae V-VII). The number of neurons expressing only c-Fos immunoreactivity (463), only PV immunoreactivity (119), or immunoreactivity for both c-Fos and PV (25) were counted in 3 sections from each of 3 animals. Within the three regions where neurons exhibiting c-Fos immunoreactivity were identified, only 25/463 cells (5%) also expressed PV. Cells co-localizing both PV and c-Fos were found mostly around the central canal with fewer double labeled cells in the intermediolateral region, and none in the dorsal horn. Qualitatively similar results were obtained when c-Fos was co-localized with GABA. These results indicate that a relatively small sub-population of neurons

activated during reflex micturition express GABAergic traits. Such cells may function to inhibit somatic motoneurons leading to a reduction in urethral pressure during micturition.

Bladder and urethral pressures evoked by microstimulation of the sacral spinal cord

The goal of this project was to determine the physiological effects in the genitourinary system of microstimulation of the sacral spinal cord. Experiments were conducted to map systematically the bladder and urethral pressures evoked by intraspinal stimulation of the sacral segments (S1-S3) in neurologically intact, chloralose anesthetized adult male cats. The bladder pressure was measured with a superpubic catheter and the urethral pressure was measured simultaneously at the level of the urethral sphincter and at the level of the penis using a two-element micromanometer. Intraspinal stimuli (1s, 20Hz, 100μA, 100μs) were applied with activated iridium microwire electrodes in ipsilateral segments and intersegmental boundaries with a 250 μm mediolateral resolution and a 200 μm dorsoventral resolution. Increases in bladder pressures were generated by microstimulation in the intermediolateral region, in the lateral and ventrolateral ventral horn, and around the central canal. Simultaneous increases in intraurethral pressure were evoked by microstimulation in the ventrolateral ventral horn, but not at the other locations. Reductions in intraurethral pressure were evoked at locations in the intermediate laminae and around the central canal. The magnitude of these pressure reductions was weakly dependent on the stimulus parameters, however, the sign and magnitude of the urethral pressure responses were dependent on the location of the pressure sensor in the urethra. Stimulation around the central canal produced bladder contractions with either no change or a reduction in urethral pressure and voiding of small amounts of fluid. These results demonstrate that regions are present in the spinal intact anesthetized animal that generate selective contraction of the bladder without activation of the urethral sphincter.

Functional Anatomy of the Urethra in the Male Cat

During our microstimulation experiments we found that the urethral pressure responses were complex and strongly dependent on the position and orientation of the sensor within the urethra. Therefore the goal of this study was to determine the anatomy of the periurethral musculature and the pressures produced by stimulation of different periurethral muscles. Anatomical and histological methods were combined with measurements of the urethral pressure profile (UPP) to investigate the functional aspects of the urethra in male cats. A silicone rubber catheter with two micro-diaphragm pressure transducers was used to measure the UPP. Gross anatomy and ultrastructure of the urethra at each segment were examined and correlated with the pressure profile data. The preprostatic urethra was composed of three layers of smooth muscle, while distal to the prostate striated muscle became predominant. Increased baseline pressure and rapid fluctuations in pressure in the postprostatic urethra and bulbourethra resulted from the function of periurethral striated musculature. The UPP was affected by the bladder pressure, repetition of the measurement, the sensor orientation in the urethra, and the type of measurement catheter. Well controlled high fidelity measurements enabled a clear correlation to be established between the features of the UPP and the anatomy of the urethra and surrounding musculature. The observations on the ultrastructural and microscopic anatomy of the urethra extend a previous description of the pelvic urethra.

Additionally, electrical stimulation was used to study the neural pathways that mediate the urethral pressure responses evoked by microstimulation of the spinal cord. Urethral pressures were measured in male cats anesthetized with alpha-chloralose using two catheter mounted pressure transducers. Pudendal nerve branches innervating the urethra were unilaterally transected and the proximal or distal end of each branch was stimulated. Pressure increases were detected in the postprostatic urethra and bulbourethra, where periurethral striated muscle was identified in histological sections. Threshold stimuli (20 Hz, 1 s train) resulted in pressure increases of 3-11 cm-H2O; supramaximal stimuli generated pressures of up to 300 cm-H2O. The pressures generated by afferent fiber activation were strongly dependent on stimulus frequency. At 2-5 Hz

the responses followed one-to-one with the stimulus and maintained a constant amplitude; at 10 Hz, the responses followed one-to-one, but the amplitude fell sharply after the first stimulus; at 20 Hz a strong onset response was followed by a weak sustained response during the stimulus train. These responses were similar to pressures evoked by microstimulation in the sacral dorsal horn, while the responses generated by efferent stimulation were similar to pressures generated by microstimulation in the sacral ventral horn. We conclude that a major part of the urethral response to spinal microstimulation is mediated by pudendal afferent and efferent nerve fibers.

Hindlimb motor responses evoked by microstimulation of the lumbar spinal cord

The goal of this project was to determine the physiological effects in the motor system of microstimulation of the lumbar spinal cord. Experiments were conducted to map systematically the hindlimb motor responses evoked by intraspinal stimulation of the lumbar segments (L5-L7) in neurologically intact, chloralose anesthetized adult cats. The isometric torque generated about the knee joint and intramuscular EMGs from knee flexors and extensors were recorded in response to intraspinal stimuli (1s, 20Hz, 100μA, 100μs) applied with activated iridium microwire electrodes. Stimuli were applied in ipsilateral and contralateral segments and intersegmental boundaries with a 250 μm mediolateral resolution and a 200 μm dorsoventral resolution to create fine grained maps. Torque maps were repeatable across experiments with a strong congruence between the spatial patterns of torque generation and similar relative magnitudes of flexion and extension torques. At L6 and L7, stimulation in the ipsilateral dorsal aspect of the cord produced strong flexion torques. Weaker flexion torques were also produced by microstimulation at the lateral aspect of the intermediate region. Stimulation in the contralateral dorsal horn produced extension torques that accommodated rapidly during the 1 s stimulus. In the ipsilateral ventral horn, extension torques were produced at locations that were lateral to locations producing flexion torques, and stronger flexion torques were produced by microstimulation over a larger area in L7 than in L6. EMG recordings indicated that microstimulation in the ipsilateral dorsal, intermediate, and medial ventral locations activated knee flexors selectively, while microstimulation in lateral ventral locations activated knee extensors selectively. Microstimulation mapping studies in the motor system demonstrated segregation of responses in the ventral horn that correlate with previous anatomical tracing studies. Stimulation in the dorsal aspect of the cord appeared to generate electrically two classical spinal reflexes: ipsilateral flexion (flexion withdrawal) and contralateral extension (crossed extension).

Surface stimulation for intraoperative localization

The goal of this project was to determine the feasibility of using surface stimulation of the spinal cord to predict the responses that would be obtained by intraspinal stimulation. In the course of our microstimulation mapping studies we routinely stimulated on the surface of the cord and the physiological responses in the genitourinary (bladder pressure, urethral pressures) and motor (knee torque, EMG in hindlimb muscles) were recorded. These data indicated that the surface evoked response is a poor predictor of the response that will be evoked by depth stimulation. The surface responses were dominated by stimulation of afferent terminals in the lateral cord and stimulation of dorsal columns in medial part of cord. There was significant variation in evoked responses response along the depth of a particular track which was not be predicted by surface stimulation. For example, in the hindlimb motor system, surface stimulation invariably generated flexion torques, while depth stimulation generated either flexion torques or extension torques dependent on the depth and mediolateral location. Further, while one may contemplate use of a multiple electrode array on the spinal cord surface to focus stimulation at a depth, Laplace's equation indicates that the maximum of the induced electric field will always be present on the surface or at discontinuities in conductivity and cannot be "focused" in depth. Therefore, surface stimulation is unlikely to provide an accurate indicator of responses that will be obtained with depth stimulation.

PUBLICATIONS

Journal Papers

Grill, W.M., B. Wang, S. Hadziefendic, M.A. Haxhiu (1998) Identification of the spinal neural network involved in coordination of micturition in the male cat. Brain Research 796:150-160.

Wang, B., W.M. Grill, N. Bhadra (1998) Functional anatomy of the male feline urethra: morphological and physiological correlations. Journal of Urology, in press.

Barbeau, H., D.A. McCrea, M.J. O'Donovan, S. Rossignol, W.M. Grill, M.A. Lemay, Tapping into spinal circuits to restore motor function. submitted to Brain Research Reviews.

Grill, W.M., B.O. Erokwu, S. Hadziefendic, M.A. Haxhiu, Supramedullary neural network controlling the bladder and urethra: a transneuronal labeling study using pseudorabies virus. submitted to J. of Comparative Neurology.

Book Chapter

Grill, W.M., R.F. Kirsch (1999) "Neural Prostheses", Encyclopedia of Electrical and Electronics Engineering, J.G. Webster, Ed., John Wiley and Sons, Inc, in press.

Conference Abstracts

Grill, W.M. and N. Bhadra (1996) Genitourinary responses to microstimulation of the sacral spinal cord. Society for Neuroscience Abstracts 22:1842.

Erokwu, B., W.M. Grill, and M.A. Haxhiu (1996) Supramedullary innervation of the urethra. Society for Neuroscience Abstracts 22:94.

Wang, B., W.M. Grill, N. Bhadra (1997) Urethral pressure responses to stimulation of afferent and efferent neurons in the pudendal nerve and sacral spinal cord of male cats. Proc. 2nd Conf. Int. Functional Electrical Stimulation Soc.

Grill, W.M., B. Wang, N. Bhadra (1997) Isometric knee torque generated by microstimulation of the lumbar spinal cord. Proc. 2nd Conf. Int. Functional Electrical Stimulation Soc.

Wang, B., W.M. Grill, N. Bhadra (1997) Feline urethral musculature and its neural control. Society for Neuroscience Abstracts 23:1522.

Grill, W.M., B. Wang, M.A. Haxhiu (1997) Identification of neurons in the sacral spinal cord of the cat involved in genitourinary regulation. Society for Neuroscience Abstracts 23:1522.

Grill, W.M., B. Wang (1997) Mapping knee torques evoked by intraspinal microstimulation. Proc. 19th Ann. Int. Conf. IEEE-EMBS.

Grill, W.M., S. Hadziefendic, B.O. Erokwu, M.A. Haxhiu (1998) Co-localization of parvalbumin and c-fos in sacral spinal neurons involved in regulation of micturition. Society for Neuroscience Abstracts 24.

Grill, W.M., B. Wang, N. Bhadra, M.A. Haxhiu (1998) Spinal neurons regulating micturition: identification and effects of stimulation. 4th Annual Meeting of the International Neuromodulation Society/3rd Conf. Int. Functional Electrical Stimulation Soc.

RECOMMENDATIONS FOR FUTURE RESEARCH AND DEVELOPMENT

The results of this work have demonstrated that microstimulation can generate coordinated physiological responses in the genitourinary and motor systems. In both cases these results were obtained in anesthetized, neurologically intact animals. Therefore an important extension of this work is conduct microstimulation mapping experiments in decerebrate and chronic spinal preparations. Further, in both cases, coordinated responses were obtained by stimulation of higher-order neurons, rather than last order motoneurons. Therefore, anatomical tracing to identify the location and rostrocaudal extent of genitourinary and motor interneurons should continue. The data to date also indicate that surface stimulation is unlikely to provide accurate predictions of the physiological responses obtained by depth stimulation. Therefore, alternative methods should be considered for intraoperative localization of neuronal populations in human. Finally, an important element of a neural prosthesis employing microstimulation is the ability to activate selectively distinct populations of neurons. An effort to design and test multiple electrode arrays and stimulus parameters for selective stimulation should be initiated. This should include a significant component in computer-based modeling as this affords to opportunity to examine excitation under highly controlled conditions that would be difficult to achieve experimentally.